PROJECT NUMBER: 1904
PROJECT TITLE: Tobacco Physiology and Biochemistry
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PERIOD COVERED: July, 1987

I. LOW NICOTINE STUDY

A. Objective: To investigate the biochemistry of the nicotine biosynthetic pathway at putrescine N-methyltransferase (PMT) and N-methylputrescine oxidase (MPO) and specifically to isolate PMT from tobacco root extracts.

B. Status: A fourth group of hydroponically grown, Burley 21 (Bu21) tobacco plants (36 plants, approximately double the previous harvest) is being processed to obtain root extracts of a 40-65% ammonium sulfate fraction (2). A phenyl-sepharose column (hydrophobic interaction column) has been successfully used with tobacco root extracts, PM 27 and PM 28. It was discovered that the addition of 1.5 N NaCl directly to the 40-60% ammonium sulfate fraction was necessary in order to bind PMT to this column. This has eliminated the time and buffer consuming process of dialysis used previously. Four separate applications of tobacco extracts (at the ammonium sulfate stage) have been made to phenyl-sepharose columns and PMT-active fractions have been pooled and stored frozen at -80°C (1,3).

A fraction from a phenyl-sepharose column was applied to a FPLC Superose 12 gel filtration column to obtain 0.05 ml fractions (see previous monthly). The PMT-activity was examined and an apparent molecular weight was estimated to be about 60 kD. The smaller sample volume did not increase the resolution, i.e., protein bands in the 40 - 60 kD molecular weight range were still present in the estimated 60 kD mw fraction. All fractions were examined by Phast system gels which were silver stained in order to visualize the proteins. New Aurodye and silver staining procedures were used successfully to detect the proteins (4).

A secondary antibody staining method using Auroprobe BL GAR, a goat antirabbit antibody, has been employed to examine PMT-active fractions. This new antibody procedure was utilized to examine PMT-active fractions eluted from a QAE-anion exchange cartridge (Cuno) and PMT-active fractions pooled and applied to a phenyl-sepharose column. Use of a silver enhancement technique along with the Auroprobe BL GAR allowed visualization of a positive cross-reaction of the phenylethanolamine methyltransferase (PNMT) antibody in conjunction with the goat antirabbit antibody to two proteins in the 55-60 kD molecular weight region (5).

Preparative gel electrophoresis was conducted on partially purified PMT-active fractions after the samples were dialyzed and freeze-dried. Two bands above 55 kD (based on the molecular weight of a glutamate dehydrogenase standard) were excised and electroeluted (6). These samples were subjected to dot-blot

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antibody staining as described above and positive reactions against PNMT were observed for both fractions (5).

C. **Plans**: The phenyl-sepharose column will be used to obtain initial purification of PMT from the ammonium sulfate fractions. Pooled fractions from phenyl-sepharose will be applied to the QAE anion exchange cartridge as the second step in the PMT-purification scheme. Preparative gel electrophoresis will be continued to obtain samples for future proposed antibody production.

D. **References**:

1. Malik, V. S. PM Notebook No. 8402.
2. Shelton, S. PM Notebook No. 8486.
3. Nakatani, H. Y. PM Notebook No. 8384.
4. Sherwood, K. R. PM Notebook No. 8416.
5. Mooz, E. D. PM Notebook No. 8296.
6. Sykes, A. PM Notebook No. 8526.

II. **OTHER PROJECT ACTIVITIES**:

Notification of the acceptance by TCRC of four abstracts submitted by members of Project 1904 for consideration has been received. Manuscripts are being revised and slides are being prepared for the presentations in October.

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